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Feces composition and manure derived methane yield from dairy cows: Influence of diet with focus on fat supplement and roughage type

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HIGHLIGHTS

- We model the methane potential from manure by diet and feces composition.
- We correlate crude fat in faces with the methane potential.
- Increasing fat in the diet increase the methane potential.

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ABSTRACT

The objective of the present study was to evaluate the effect of dairy cow diets on feces composition and methane (CH_4) potential from manure with emphasis on fat level and roughage type and compare these results with the corresponding enteric CH_4 emission. In experiment 1 six different diets, divided into two fat levels (low and high) and three different roughage types (early cut grass silage, late cut grass silage and maize silage), were used. The high fat level was achieved by adding crushed rapeseed. In experiment 2, the influence of increasing the fat level by using three different types of rapeseed: rapeseed cake, whole seed and rapeseed oil against a low fat ration with no rapeseed fat supplementation was studied. The diet and fat level had a significant influence on feces composition and CH_4 yield. In general, ultimate CH_4 yields (B_0) were 8–9% higher than the present international default values for diets without extra fat and in feces from diets with extra fat supply the yield was 25–31% higher. It was possible to predict the B_0 value from feed and feces characteristics; in fact, the best correlation was obtained by including both feed and feces characteristics. Addition of crude fat to diets to dairy cows reduced enteric CH_4 emission but at the same time increased CH_4 potential from feces both in terms of organic matter in feces and dry matter intake which might lead to increasing emissions unless proper manure handling such as anaerobic digestion is included. Without subsequent anaerobic digestion to produce energy the positive effect achieved at cow level could be counteracted by increasing manure emissions.

1. Introduction

The environmental benefits of using manure in biogas plants is much higher than for any other substrate due to the combined effect of production of methane (CH_4) as a non-fossil fuel, and the corresponding reduction in the emissions of methane to the atmosphere from unwanted anaerobic degradation during slurry storage and application on the fields (Sommer et al., 2004). At the same time concern on greenhouse gas (GHG) emission from livestock production is growing due to CH_4 emissions associated with manure storage and enteric fermentation. Approaches to diminish ruminant enteric fermentation by changes in forage type and quality and supplementation with fat have been adopted and preliminary results indicate a positive effect. However the effects of changing diets in terms of biogas potential and potential CH_4 losses from manure storage has not been sufficiently studied. Knowledge on the variation in manure CH_4 potential losses during storage originating from the diet is scarce. Accordingly, there is a need for

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updated estimates of biogas potential of manure from cattle fed different diets, as national inventories on the biogas potentials from manure today are based on very rough outdated estimates that are not corrected for changes in feeding strategy. A more precise documentation of the biogas potential of manure will provide an improved decision tool for dimensioning, projecting and economic budgeting of new biogas installations based primarily on manure. Ultimate biogas yield of manure (B_o) during indefinite anaerobic digestion is the value that together with an emission factor is used to determine the amount of CH_4 emitted during storage of untreated manure. Precise knowledge of B_o is a pre-requisite to predict CH_4 emission or production by anaerobic digestion either during manure storage as slurry or in a biogas digester.

Protocols for estimating CH_4 emissions from manure management have been set out by the Intergovernmental Panel on Climate Change (IPCC, 2006) and Denmark, like most European countries, uses the IPCC tier2 methodology (Nielsen et al., 2013). This protocol estimates CH_4 emission using volatile solids (VS) excreted by the animals, CH_4 conversion factor (MCF) and ultimate CH_4 yield (B_0). Denmark's National Inventory (Nielsen et al., 2013) uses national values to calculate VS excreted, a MCF of 10% for slurry management and the default values provided by the IPCC (2006) for B_0 (240 L CH_4/kg VS for dairy cattle manure). Nevertheless, IPCC (2006) recommends expanding the representativeness of the default values using specific country values, especially for livestock in tropical regions and when varying diet regimen.

Variations in diet regimen can be used as mitigation strategies for ruminant enteric CH_4, like fat supplementation, feed additives, increments in concentrate inclusion levels and improving roughage quality (Gerber et al., 2013). The addition of fat to the diet considerably reduces enteric CH_4 emission in ruminants, not only because of the inhibitory effect on rumen methanogenesis (Martin et al., 2008), but also because of the potential reduction in fiber digestibility (Boadi et al., 2004). However, the effect of fat in reducing enteric CH_4 emission is affected by the amount and the type of fat added (degree of saturation) and its availability in the rumen (Boadi et al., 2004) and furthermore a negative effect on the dry matter intake of the animal may also be expected at high levels of fat supplementation in the diet (Weisbjerg et al., 2008) thereby partly negating the potential positive effect of fat supplementation on enteric CH_4 emission. The roughage type (maize *vs*. grass) and the maturity at harvest also effects enteric CH_4 emission (Brask et al., 2013a). Harvest in an earlier stage of grass maturity has often been proposed as a strategy to decrease enteric CH_4 production, as less mature grass is accompanied by lower neutral detergent fiber (NDF) content in grass and higher digestibility (Van Soest, 1994; Barrière et al., 2005).

Although different feeding strategies can reduce enteric CH_4 emission from ruminants, the effect on subsequent manure-derived CH_4 production and on B_0 remains unclear. In fact, most of these strategies are based on variations in the digestibility of nutrients; therefore, by using these strategies alterations on biodegradable organic content in the manure can be expected, and consequently on B_0. Nevertheless, increases in organic matter biodegradability could be desirable in animal manure when it is used for biogas production. Using this technology, organic matter is transformed into CH_4 which serves as a renewable energy source, reducing simultaneously the emission of CH_4 to the atmosphere during slurry management (Sommer et al., 2004).

The objective of the present work was to evaluate the effect of different strategies to mitigate enteric CH_4 emission, including different types of rapeseed (RS[1]) as fat source and varying roughage type and harvest time of silage, on the subsequent CH_4 potential from manure and comparing these results with the corresponding enteric CH_4 emission. Furthermore the objective was to develop a model for the CH_4 potential of feces by knowing diet and/or feces characteristics.

[1] RS: Rapeseed.

Table 1
Diet composition used in experiment 1 and 2.

Diet	Dry matter	Organic matter	Crude protein	Crude fat	Fatty acids	Neutral detergent fiber	Gross energy
	% of fresh matter	% of DM					MJ/kg DM
Experiment 1							
[1] EG	51.2	90.8	20.9	3.7	2.9	30.4	17.9
EG + fat	52.0	91.5	20.4	6.4	5.4	29.9	18.5
[2] LG	42.1	92.2	18.0	3.1	2.3	40.7	18.0
LG + fat	42.8	92.4	17.8	5.8	4.8	39.1	18.7
[3] M	38.8	94.8	16.4	3.4	2.9	35.5	18.5
M + fat	40.2	94.9	15.5	6.2	5.6	33.7	19.1
Experiment 2							
[4] RM	47.9	93.4	16.9	3.5	2.6	33.2	18.4
RS Cake	50.0	93.7	17.1	5.5	4.3	32.8	18.9
Whole RS	49.2	93.9	16.8	6.2	5.0	32.6	19.1
RS Oil	49.4	93.9	17.1	6.5	5.3	32.2	19.1

Notes: [1] EG: early harvest grass-clover silage; [2] LG: Late harvest grass-clover silage; [3] M: Maize silage; [4] RM: rapeseed meal.

2. Material and methods

2.1. Animals and diets

Two experiments were carried out. In the first experiment (**experiment 1**) six different diets were formulated to test different fat levels (3.7–6.4% of DM) in combination with three different roughage types (early cut grass silage, late cut grass silage and maize silage). High fat level was achieved by adding crushed RS. The different diets used in experiment 1 were:

- ***EG***[2]: diet formulated using early harvest primary growth grass-clover silage and concentrate low in fat
- ***EG + fat***: diet formulated using early harvest primary growth grass-clover silage and concentrate high in fat
- ***LG***[3]: diet formulated using late harvest primary growth grass-clover silage and concentrate low in fat
- **LG + fat**: diet formulated using late harvest primary growth grass-clover silage and concentrate high in fat
- **M**[4]: diet formulated using maize silage and concentrate low in fat
- **M + fat**: diet formulated using maize silage and concentrate high in fat

In the second experiment (**experiment 2**) addition of three different types of RS: rapeseed cake (*RS Cake*), whole seed (*Whole RS*) and rapeseed oil (*RS Oil*) were evaluated against no rapeseed fat supplementation. The different diets used in experiment 2 were

- **RM**[5]: control diet with 190 g/kg of dry matter (DM) formulated using RS meal (4% fat)
- **RS Cake**: diet formulated using 62 g/kg of DM of RS meal (4% fat) and 156 g/kg of DM of RSC (17% fat)
- **Whole RS**: diet formulated using 149 g/kg of DM of RS meal (4% fat) and 69 g/kg of DM of WRS (48% fat)

[2] EG: diet formulated using early harvest primary growth grass-clover silage.
[3] LG: diet formulated using late harvest primary growth grass-clover silage.
[4] M: diet formulated using maize silage.
[5] RM: Control diet formulated with rapeseed meal.

- **RS Oil**: diet formulated using 184 g/kg of DM of RS meal (4% fat) and 33 g/kg of DM of rapeseed oil (RO)

Chemical composition of the diets used in both experiments is shown in Table 1. In both experiments, lactating Danish Holstein dairy cows (6 cows in experiment 1 and 4 cows in experiment 2) were assigned to the different diets over 4 different periods of 4 weeks. During the first two weeks of each period animals were allowed to diet adaptation, which from other studies seems as an appropriate stabilization period (Huhtanen and Hetta, 2012); from day 15–19 twelve samples of feces were obtained from the rectum of each animal and pooled as described in Brask et al. (2013a).

In this work results concerning feces composition and CH_4 production from manure derived from the different diets are presented and discussed. Diet formulation, milk production, nutrient digestibility and enteric CH_4 production derived from experiment 1 and 2 have been previously published in Brask et al. (2013a) and Brask et al. (2013b), respectively.

2.2. Methane yield

Ultimate CH_4 yield (B_0) was determined in feces collected from experiment 1 and 2 in a batch assay (in 0.5 L bottles) according to the protocol defined by Møller et al. (2004). Anaerobic sludge was collected from the anaerobic digester plant at Research Centre Foulum (Aarhus University, Denmark) and pre-incubated during 15 days at 35 °C in order to deplete the residual biodegradable organic material (degasification). Each manure sample was digested in triplicates. Standard deviations were in all experiments less than 6% of the mean of triplicates. Additionally three controls containing anaerobic sludge-only were digested in order to determine the anaerobic sludge endogenous CH_4 production which was subtracted from the CH_4 produced by the feces at each biogas sampling day.

After filling, each bottle was sealed with butyl rubber stoppers and aluminum crimps and the headspace was flushed with pure N_2 for 2 min. All bottles were then incubated for 90 days at 35 °C. Biogas volume in all bottles was measured by inserting a needle connected to a tube with inlet to a column filled with acidified water (pH < 2) through the butyl rubber. The biogas produced was calculated by the water displaced until the two pressures (column and headspace in bottles) were equal. Biogas samples were taken every time the total volume of biogas was measured to determine the CH_4 and CO_2 concentration in the biogas.

The biogas yield and the CH_4 yield (BMP[6]) at 30, 60 and 90 days were expressed as the cumulative production (mL) per gram of VS from the slurry introduced to the bottles. Biogas yield at 30 and 60 and 90 days are designed as $Biogas_{30}$, $Biogas_{60}$ and $Biogas_{90}$ respectively. The BMP at 30, 60 days are designed as BMP_{30}, BMP_{60}; the BMP after 90 days is regarded as the B_0.

2.3. Analytical methods

Diets from both experiments were analyzed to determine contents of crude protein (CP), crude fat (CF), gross energy (GE), NDF and fatty acids (FA). The analytical methods are described in Brask et al. (2013b). As these authors reported, CF was determined by measuring extracted lipids with petroleum ether (Soxtec 2050, Foss analytical, Hillerød, Denmark) after hydrolyzing with HCl (Stoldt, 1952). Gross energy was analyzed using an adiabatic bomb calorimeter (Parr 6300 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, IL). The NDF content in diet was determined following the procedure described Mertens (2002) and corrected for ash. Fatty acids in feed were analyzed following the procedure described by Jensen (2008).

Fresh feces were analyzed for dry-matter (DM), volatile solids (VS) and ash content (APHA, 2005). Volatile fatty acids (VFA) were determined using gas chromatography equipped with a flame ionization detector (HP 68050 series Hewlett Packard) (APHA, 2005). The VFAs determined were: acetic acid, valeric acid, butyric acid, propionic acid, 2-methyl propionic acid, and methyl butyric acid + isovaleric acid. Total kjeldahl nitrogen (TKN), was determined according to the Kjeldahl method (APHA, 2005). Total ammonia nitrogen (TAN) was determined by using photometric kits (Spectroquant® kit, Merk, USA).

Crude fat and fiber fractions (neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin) were determined from feces after the samples were dried (60 °C during 48 h) and milled (0.8 mm). Fiber fractions were determined according to the Van Soest procedure (Van Soest, 1991) and corrected for ash. Crude fat concentration in feces was determined by measuring extracted lipids with petroleum ether as described previously.

CH_4 and CO_2 concentration in the biogas were analyzed on a Perkin Elmer Clarus 500 gas chromatograph equipped with a thermal conductivity detector according to Sutaryo et al. (2012). The temperatures of injection port, oven, filament and detector were 110 °C, 40 °C, and 150 °C, respectively. The carrier gas was helium with a flow rate of 30 mL min^{-1}.

2.4. Calculations and statistical analysis

In order to compare slurries on the same basis, ash, hemicellulose, cellulose, protein, lignin and VFA content were expressed as percentage of DM. The hemicellulose content in feces was calculated as the difference between NDF and ADF, cellulose content in feces was calculated as the difference between ADF and ADL, and lignin content was assumed to be equal to ADL. Protein content was determined as (TKN − TAN) × 6.25.

Fecal CH_4 potential (MP_{DMin}) per kg DM intake was determined according to Equation (1):

$$MP_{DMin}\left(\frac{LCH_4}{Kg\ DM_{intake}}\right) = \frac{B_0\left(\frac{LCH_4}{Kg\ VS}\right) \times \text{Fecal flow}\left(\frac{Kg\ DM}{day\ cow}\right) \times VS_{feces}\left(\frac{kg\ VS}{Kg\ DM}\right)}{\text{Dry matter intake}\left(\frac{Kg\ DM}{cow\ day}\right)} \quad (1)$$

Fecal flow and DM intake were extracted from Brask et al. (2013a) and Brask et al. (2013b).

Data were analyzed using SAS® System Software (SAS Inst. Inc., Cary, NC). Differences among diets in feces composition, total biogas production, B_0, and CH_4 concentration in biogas were tested by analysis of variance using the GLM procedure of SAS. In

[6] BMP: Methane potential at different incubation times.

all cases, the diet was considered as the main factor in the models.

The relationship between feces and diet composition and biogas and BMP was studied using a correlation analysis (PROC CORR) of SAS® System Software (SAS Inst. Inc., Cary, NC). Three correlation analyses were performed using data from experiment 1, experiment 2 and combining experiment 1 and 2. Multiple regression analysis with two automated subset search algorithms: maximum R-squared improvement (PROC REG/MAXR SELECTION) and (PROC REG/FORWARD SELECTION) of SAS, were also used combining experiment 1 and 2 databases. Three groups of variables were used in the multiple regression analysis: diet composition variables (diet model 2), feces composition variables (feces model 3) and combining diet and feces variables (combined model 4) in order to estimate biogas and BMP.

$$BMP = Con + a \times GE + b \times CF + c \times FA + d \times NDF + e \times CP \tag{2}$$

$$BMP = Con + f \times Cel + g \times Hem + h \times Lipid + i \times VFA + j \times Prot + k \times Lig \tag{3}$$

$$BMP = Con + a \times GE + b \times CF + c \times FA + d \times NDF + e \times CP + f \times Cel + g \times Hem + h \times Lipid + i \times VFA + j \times Prot + k \times lig \tag{4}$$

Where *Con* is a constant, and the explanatory variables are: *GE*, is the gross energy in diet, *CF* is the crude fat in diet, *FA* is the fatty acids in diet, *NDF* is the neutral detergent fiber in diet; *Cel* is the cellulose in feces, *Hem* is the hemicellulose content in feces, *Lig* is the lignin content in feces, *VFA* is the volatile fatty acids in feces and *Prot* is the protein content in feces. Parameters are expressed as percentage of DM except GE (MJ/kgDM).

Additionally, the relation between enteric CH_4 production and CH_4 yield from feces expressed in terms of L CH_4/kg DM intake was studied. Enteric CH_4 production, in terms of L CH_4/kg DM intake, for experiment 1 and 2 was calculated based on figures from Brask et al. (2013a) and Brask et al. (2013b), respectively.

Table 3
Average biogas production, B_0, and BMP obtained in feces in experiment 1 and 2. Statistical significant differences among diets ($P < 0.05$) are indicated by different superscripts (a through d) within each row.

Diet	Biogas			BMP		
Day	30	60	90	30	60	90 (B_o)
Experiment 1						
[1] EG	302^{cd}	390^{c}	406	192^{cd}	248^{abc}	258^{ab}
EG + fat	349^{abcd}	464^{abc}	485	228^{abc}	303^{a}	316^{a}
[2] LG	279^{d}	372^{cd}	388	172^{d}	230^{c}	240^{b}
LG + fat	328^{bcd}	447^{abc}	495	207^{bcd}	282^{abc}	312^{a}
[3] M	323^{bcd}	450^{abc}	454	187^{cd}	261^{abc}	263^{ab}
MC + fat	354^{abcd}	495^{a}	506	212^{abcd}	296^{ab}	302^{ab}
Experiment 2						
[4] RM	364^{abc}	396^{bc}	424	231^{abc}	251^{bc}	269^{ab}
RS Cake	383^{ab}	412^{abc}	440	243^{ab}	261^{abc}	279^{ab}
Whole RS	417^{a}	447^{abc}	475	264^{a}	283^{ab}	301^{ab}
RS Oil	421^{a}	451^{ab}	480	261^{a}	280^{abc}	298^{ab}
†SEM	21.2	22.3	27.9	12.8	13.5	17.1
P value	<0.05	<0.05	‡ns	<0.05	<0.05	<0.05

Notes: [1] EG: early harvest primary growth grass-clover silage; [2] LG: Late harvest primary growth grass-clover silage; [3] MC: Maize silage; [4] RM: rapeseed meal; † SEM: standard error of the mean; ‡ns: not significant ($P > 0.05$).

3. Results and discussion

3.1. Feces composition

Table 2 shows the average feces composition obtained from the different diets tested in experiment 1 and 2 and the statistical significant differences ($P < 0.05$) among treatments. As shown, feces obtained in experiment 1 in general showed a higher variability in chemical composition than feces obtained in experiment 2. In experiment 1, the greatest differences in feces composition were observed between *EG and M*; although statistical significant differences ($P < 0.05$) were also observed for VFA concentration and CF content in feces between *EG* and *LG*.

Feces obtained from *EG* diet showed lower hemicellulose content than feces from *M* diet at both fat levels. Although cellulose content in feces did not show statistical significant differences among treatments, feces from *EG* and *EG + fat* diets showed the lowest cellulose content. This lower cellulose and hemicellulose content found in feces from early grass could be related to a lower lignification, because of the lower NDF content in *EG* than in *LG* and

Table 2
Average feces composition in experiment 1 and 2. Parameters are expressed as percentage of dry matter (DM) (%) except noted. Statistical significant differences among diets ($P < 0.05$) in each experiment are indicated by different superscripts (a through c) within each row.

Diet	Dry matter	Ash	Cellulose	Hemi-cellulose	Lignin	Volatile fatty acids[5]	Crude protein	Crude fat
	(%)	% Of dry matter						
Experiment 1								
[1] EG	14.6^{a}	14.1^{a}	19.6	15.0^{b}	18.4^{ab}	2.0^{a}	16.4^{a}	7.4^{abc}
EG + fat	14.2^{a}	13.8^{a}	19.9	11.4^{b}	19.2^{a}	1.4^{ab}	14.3^{ab}	12.3^{a}
[2] LG	13.4^{a}	11.6^{ab}	22.7	20.6^{ab}	17.9^{ab}	1.3^{b}	11.3^{ab}	4.7^{c}
LG + fat	11.8^{b}	11.5^{ab}	22.7	20.7^{ab}	16.6^{ab}	1.0^{b}	12.2^{ab}	7.9^{abc}
[3] M	14.3^{ab}	9.6^{b}	25.8	26.6^{a}	14.1^{ab}	1.0^{b}	9.6^{ab}	3.7^{c}
M + fat	15.6^{a}	9.3^{b}	25.9	25.7^{a}	13.4^{b}	1.0^{b}	8.8^{b}	7.4^{abc}
Experiment 2								
[4] RM	13.7^{ab}	12.3^{ab}	20.3	21.3^{a}	17.3^{ab}	1.3^{b}	12.0^{ab}	4.8^{c}
RS Cake	14.8^{a}	13.4^{a}	21.9	20.5^{ab}	16.4^{ab}	0.9^{b}	10.7^{b}	7.4^{bc}
Whole RS	14.5^{a}	11.7^{ab}	22.3	21.6^{a}	15.0^{ab}	0.8^{b}	10.5^{b}	8.2^{abc}
RS Oil	14.7^{a}	11.5^{ab}	21.1	21.1^{a}	15.4^{ab}	0.8^{b}	11.3^{b}	10.7^{ab}
†SEM	0.63	0.73	1.58	1.6	1.18	0.18	1.81	1.27
P value	<0.05	<0.05	‡ns	<0.05	<0.05	<0.05	<0.05	<0.05

Notes: [1] EG: early harvest primary growth grass-clover silage; [2] LG: Late harvest primary growth grass-clover silage; [3] MC: Maize silage; [4] RM: rapeseed meal; [5]Measured in dried samples. † SEM: standard error of the mean; ‡ns: not significant ($P > 0.05$).

M diets. Additionally, a higher fiber digestibility could explain these differences. In fact, Brask et al. (2013a) found a higher total tract digestibility of organic matter and NDF in *EG* diets compared with *LG* and *M* diet. The lignin content in feces from *EG* diet was significantly higher than that obtained in feces from *M* + *fat* diet. This might be caused by the same reasons as observed by Hindrichsen et al. (2005), who found a higher lignin content in slurries from cows fed with apple pulp diet compared to slurries from cows fed with oat hull. This fact was explained by the poor distribution of the lignin content in easily biodegradable substrates, such as apple pulp; which allows animals to digest the non-lignified part of the fibers, increasing therefore the indigestible part in feces.

Feces obtained from *EG* and *EG* + *fat* diet showed the highest VFA content. The individual VFA concentration in feces (data not shown) from *EG* diet showed the highest value for all individual VFA except for butyric acid, where the difference among treatments did not reach significance.

Crude fat content was higher in feces from *EG* + *fat* diet compared with crude fat in feces from *LG* and *M* diets.

In experiment 2, the only statistical significant difference ($P < 0.05$) was found in the CF content in feces. The CF content in feces from *RM* diet was lower than that obtained in feces from *RS Oil* diet (Table 2). These results were in accordance with the findings by Brask et al. (2013b), where nutrient digestibility was unaffected by change in diet, except for the fat flow, which increased in animals with fat-supplemented rations.

3.2. Biogas and methane yield

The biogas yield and methane yield (BMP) from feces are given in Table 3 showing that there were differences ($P < 0.05$) among diets. The highest biogas yield during short time digestion (30 days) was obtained in feces from *Whole RS* and *RS Oil* diets. Biogas yield after 60 days in feces from *MC* + *fat* diet was higher than the yield obtained in feces from *RM* diet in experiment 2 and *EG* and *LG* diets in experiment 1. The long term biogas yield (90 days) did not show statistical significant differences among treatments.

The BMP showed similar trends as did the biogas yield, especially after 30 and 60 days. The highest BMP_{30} was obtained in feces from *Whole RS* and *RS Oil* diets, meaning an increase of 14.6% and 13.1% compared with BMP_{30} obtained in feces from *RM* diet. This increase in BMP_{30} in feces from diets with a high fat content is important in cases of anaerobic digestion of the slurry, since retention times between 20 to 30 days are often used in continuous stirred mesophilic anaerobic digesters in countries as Denmark. This indicates that organic matter from feces with a high content in CF, due to a higher fat content in their corresponding diets, has a higher CH_4 yield after 30 days and therefore, feces from diet with fat supplementation will have a higher value and improve economic performance of the biogas plant. This might be explained by the fact that lipids have a higher theoretical CH_4 yield than carbohydrates and proteins (Angelidaki and Sanders, 2004). However, differences were partially reduced during longer digestion time after 60 and 90 days, indicating therefore that, organic matter from feces with a high CF content is faster converted into CH_4 compared to organic matter in feces with a low CF content.

During long time digestion (BMP > 60days) the feces from high fat diets showed higher BMP than feces from low fat diets; although statistical significant differences between fat levels within the same roughage type were only obtained within *LG* diets. The highest ultimate methane yield (B_0) was obtained in feces from *EG* + *fat* diet but only being statistical significant higher than the yield obtained in feces from *LG* diet. In experiment 2 the highest B_0 was obtained in feces from diets with extra fat (*RS Cake*, *Whole RS*, *RS Oil*) compared to the control (*RM*) as was the case in experiment 1.

The ultimate CH_4 yield of dairy cattle manure has been determined in several other studies (Hill, 1984; Møller et al., 2003, Amon et al., 2007). In the study by Amon et al. (2007) the CH_4 yield after 42 days was 125–159 L CH_4/kg VS and in the study by Møller et al. (2004) the CH_4 yield after 100 days was 100–207 L CH_4/kg VS. In general the earlier studies show a much lower yield than found in the present study. It thus seems that today's diets, feeding practices, higher dry-matter uptake and higher milk yields per cow result in considerably higher yields than found in former studies and especially when extra fat is supplemented to the diet. But also standard diet without extra fat supply gives higher yields than found previously.

Comparing to the IPCC default value (IPCC, 2006) which is 240 L CH_4/kg VS in dairy cattle manure this study indicates higher B_o values except for the diet from LG feces which is the diet with the lowest digestibility (240 L CH_4/kg VS). Early grass and Maize diets resulted in respectively 258 L CH_4/kg VS and 263 L CH_4/kg VS, which is 8–9% higher than the IPCC default value and adding fat to the diet increased B_0 from feces 25–31%. These findings indicate that emissions from manure could be higher than earlier calculated, especially when adding fat to the diet and at the same time the CH_4 potential from cattle manure used for biogas production seems to have a higher potential yield than assumed before.

In the present study we have assumed that we can extrapolate the results found in feces to manures without taking the urine fraction in consideration. Urine is hydrolyzed to inorganic nitrogen already during housing of the animal and there will be no energy available in the urine fraction for biogas production. However feed losses and bedding ending up in the manure will have an influence on the biogas potential but since the aim of our study is to assess the impact of feeding on the methane yield in the manure itself we have not included other factors since they will be very much dependent on housing systems and management. Water spillage to the manure system will also have a large influence on the volumetric biogas potential but the amount of water lost to the manure system will also depend on housing system and management and is thus not included in the study.

Table 4
Simple correlation analysis (*R*) among biogas, methane yield, diet and feces composition in experiment 1, experiment 2 and both experiments (experiment 1 + 2).

		Diet composition			Feces composition		
		Fatty acids	Gross energy	Crude fat	Lipid	Lignin	Volatile fatty acids
Experiment 1	$Biogas_{30}$	0.92^{**}	0.85^{*}	0.88^{*}	0.59^{ns}	-0.35^{ns}	-0.50^{ns}
	$Biogas_{60}$	0.86^{*}	0.93^{**}	0.80^{ns}	0.40^{ns}	-0.55^{ns}	-0.64^{ns}
	$Biogas_{90}$	0.91^{*}	0.93^{**}	0.89^{*}	0.48^{ns}	-0.44^{ns}	-0.66^{ns}
	BMP_{30}	0.93^{**}	0.61^{ns}	0.95^{**}	0.88^{*}	0.08^{ns}	-0.23^{ns}
	BMP_{60}	0.96^{**}	0.80^{ns}	0.95^{**}	0.73^{ns}	-0.18^{ns}	-0.45^{ns}
	B_0	0.96^{**}	0.73^{ns}	0.95^{**}	0.79^{ns}	-0.10^{ns}	-0.37^{ns}
Experiment 2	$Biogas_{30}$	0.94^{ns}	0.93^{ns}	0.93^{ns}	0.91^{ns}	-0.97^{*}	-0.92^{ns}
	$Biogas_{60}$	0.93^{ns}	0.91^{ns}	0.92^{ns}	0.91^{ns}	-0.97^{*}	-0.91^{ns}
	$Biogas_{90}$	0.93^{ns}	0.91^{ns}	0.91^{ns}	0.91^{ns}	-0.96^{*}	-0.90^{ns}
	BMP_{30}	0.94^{ns}	0.94^{ns}	0.93^{ns}	0.85^{ns}	-0.99^{**}	-0.94^{ns}
	BMP_{60}	0.93^{ns}	0.92^{ns}	0.92^{ns}	0.85^{ns}	-0.99^{**}	-0.93^{ns}
	B_0	0.92^{ns}	0.92^{ns}	0.91^{ns}	0.84^{ns}	-0.99^{**}	-0.92^{ns}
Experiment 1 + 2	$Biogas_{30}$	0.64^{*}	0.84^{**}	0.69^{*}	0.49^{ns}	-0.36^{ns}	-0.69^{*}
	$Biogas_{60}$	0.83^{**}	0.72^{*}	0.75^{*}	0.49^{ns}	-0.57^{ns}	-0.53^{ns}
	$Biogas_{90}$	0.892^{**}	0.79^{**}	0.85^{**}	0.56^{ns}	-0.50^{ns}	-0.60^{ns}
	BMP_{30}	0.63^{ns}	0.75^{*}	0.70^{*}	0.58^{ns}	-0.17^{ns}	-0.59^{ns}
	BMP_{60}	0.93^{**}	0.69^{*}	0.89^{**}	0.73^{*}	-0.28^{ns}	-0.45^{ns}
	B_0	0.93^{***}	0.75^{*}	0.93^{**}	0.79^{*}	-0.23^{ns}	-0.50^{ns}

Notes: The statistical significance is marked as follows: $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$, ^{ns}: not significant ($P > 0.05$).

3.3. Simple and multiple regression analysis

Table 4 shows the calculated parameters from the correlation analysis between feces and diet composition, and biogas and BMP using data from experiment 1 and 2. In experiment 1, Biogas and BMP were mainly correlated with diet composition, especially with CF, FA and GE, while only short term gas yield (BMP30) resulted in significant correlation with lipid content in feces. However in experiment 2, biogas and BMP in contrary had the strongest correlation with feces composition, which might be explained by higher homogeneity in diets among treatments except for CF and fatty acids. Nevertheless, although statistical significant differences were not obtained, FA, GE and CF also showed a good coefficient of determination (R^2) with biogas and BMP in experiment 2. The component that showed the strongest correlation to biogas and BMP in experiment 2 was lignin content in feces with a negative relationship, meaning that higher lignin content in feces resulted in a lower biogas and BMP production. This comply well with findings from Triolo et al. (2011), where a negatively correlation between lignin content in pig and cattle slurry and BMP was found. Triolo et al. (2011) developed a model to estimate BMP in animal slurries and energy crops with lignin content as main parameter to explain BMP variability. In experiment 1 however, this strong negative correlation between lignin content and BMP was not found.

Combining experiment 1 + 2, diet composition, especially GE, CF and FA, prevailed as the most important factor influencing biogas and BMP production from feces. The relationship between energy and fat content in the diet and biogas and BMP production from feces was positive, meaning that high energy and fat content in diets and feces promoted CH_4 production from feces. Fig. 1 shows the linear relationship between CF content in diets and BMP (a) and CF content in feces and BMP (b). There was a strong correlation between BMP and CF content in both diets and feces, indicating that increases in CH_4 yield, could be expected in dairy cow feces when CF in diets is increased. The correlation between crude fat in diets and BMP is increasing with increased digestion time. Lipid composition in the feces was the only feces parameter with significantly affected BMP when combining both experiments. It is known from other studies that a high content of lipid will have a positive influence on gas production but when exceeding certain limits there can also be a detrimental effect on methanogenic

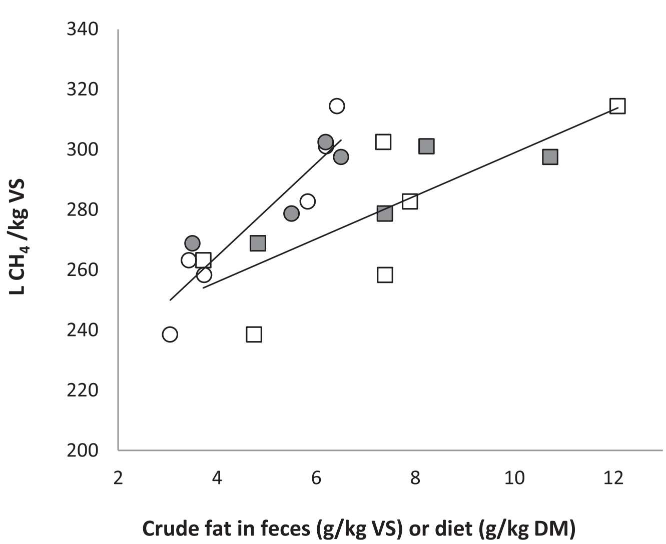

Fig. 1. Influence of crude fat in feces (□) or diet (○) on the ultimate methane yield (B_o). Experiment 1 (filled data labels) and experiment 2 (unfilled data labels). Diet ($y = 15.41x + 202.97$, $R^2 = 0.86$). Feces ($y = 7.16x + 227.37$, $R^2 = 0.62$).

bacteria through direct inhibition, sludge flotation, washout and through digester foaming (Long et al., 2012). However the lipid concentrations present in the feces in our study is below the level causing negative effect on the AD process. In order to find a model to estimate BMP from dairy cattle manure, a multiple correlation analysis was made using data from both experiments. Three groups of variables were used: diet composition variables (diet model), feces composition (feces model) and combining diet and feces variables (combined model). Table 5 shows the factors to put in the models found by multiple correlation analysis among biogas and BMP and diets and feces composition. Using diet variables biogas and BMP can be predicted by using CF, GE and FA, which is parameters most farmers knows. In the feces model, biogas and especially BMP can be predicted using a combination of lipid, lignin and VFA content which significantly increase R^2 compared to the simple correlation analysis. However using parameters both from the diet and feces composition can significantly improve the model. This means that proper estimation of the BMP is possible using feed data and measuring a few parameters in the feces.

3.4. Enteric methane and methane potential

The relation between enteric CH_4 production and CH_4 production from feces was calculated (Fig. 2). As shown, a negatively relationship was obtained between enteric CH_4 emission and CH_4 production from feces. This means that the addition of fat to diets of ruminants to decrease enteric CH_4 emission could increase CH_4 production from feces in terms of DM intake, and therefore increasing manure derived CH_4 emissions unless the manure is digested in a biogas plant prior to storage. Similar results was found by Külling et al. (2002) who observed that the reductions in enteric CH_4 emission by adding lauric acids to cow diets were followed by increased CH_4 emission during storage, due to a higher excretion of degradable fiber in the slurry. However, results from Hindrichsen et al. (2005) and Klevenhusen et al. (2011) indicate that increased CH_4 emission from the slurry is not always a consequence of reduced enteric CH_4 emissions. Moreover CH_4 emission from slurry is a result of increased biodegradability of organic matter (OM) and increased CH_4 emission from slurry could only be expected when diet modifications are followed by increased fecal OM biodegradability, as was the case in this study due to differences in crude fat in feces.

Therefore, the consequences on CH_4 emissions from manure should be considered when reducing enteric CH_4 emission in ruminants. Nevertheless CH_4 emission from the manure could be more easily avoided than enteric CH_4 emission from animal through appropriate manure managements. In fact, if reductions in enteric emission are coupled with a biogas plant it could result in a win–win situation since reductions in enteric fermentation mostly will lead to manure with higher gas potential and thus increased economic revenue for farmers if slurry is used for biogas production. However more thorough economical evaluations are needed to make an overall assessment on the economy on coupling measures on reduction of enteric fermentation with biogas production, including cost of changing the diet, influence on milk production etc. Furthermore the fate of nitrogen, potential for N_2O emissions, soil carbon storage/sequestration have not been studied and including this in a total GHG balance might change the overall picture.

4. Conclusions

Results in this study indicate that fat addition and roughage type in dairy cow diets has significant influence on feces composition and the CH_4 yield from the corresponding feces. Ultimate CH_4

Table 5
Regression coefficients and coefficient of determination (R^2) of the predictive equations obtained from the multiple correlation analysis between biogas or methane yield and diet and/or feces composition. Only correlations showing statistical significance ($P < 0.05$) are shown.

		Con	GE	CF	FA	NDF	CP	Cel.	Hem.	Lignin	Lipid	VFA	Prot.	R^2
		Diet						Feces						
Diet Model	$Biogas_{30}$	−1273	87											0.7954
	$Biogas_{60}$	375		−83	116									0.8943
	$Biogas_{90}$	341			28									0.7027
	BMP_{30}	−756	52											0.8708
	BMP_{60}	201			16									0.8553
	B_0	210			17									0.8028
Feces Model	$Biogas_{30}$	1646						−22	−7	−35		−83		0.7445
	$Biogas_{60}$													‡ns
	$Biogas_{90}$	616							−15		11			0.9285
	BMP_{30}	1076						−15	−5	−21		−59		0.9167
	BMP_{60}	657						6			8			0.8328
	B_0	316								−6	8			0.8181
Combined Model	$Biogas_{30}$	116				−6.31		−12		−8		−107		0.9925
(diet and feces)	$Biogas_{60}$	1430	99.6	−192	201							−114	24	0.9904
	$Biogas_{90}$	481					−46	14			15		30	0.9442
	BMP_{30}	658			12	−4		−10				5	−69	0.9935
	BMP_{60}	227					−24	8			10		17	0.9753
	B_0	2097	−81		27	−4	−18					−60	10	0.9943

Notes: Con: constant, GE_{diet}: gross energy in diet, CF_{diet}: crude fat in diet, FA_{diet}: fatty acids in diet, NDF_{diet}: neutral detergent fiber in diet; Cel_{feces}: cellulose in feces, Hem_{feces}: hemicellulose content in feces, $lignin_{feces}$: lignin content in feces, VFA_{feces}: volatile fatty acids in feces, $lipid_{feces,}$: lipid content in feces, $Prot_{feces}$: protein content in feces, ‡ns: not significant ($P > 0.05$).

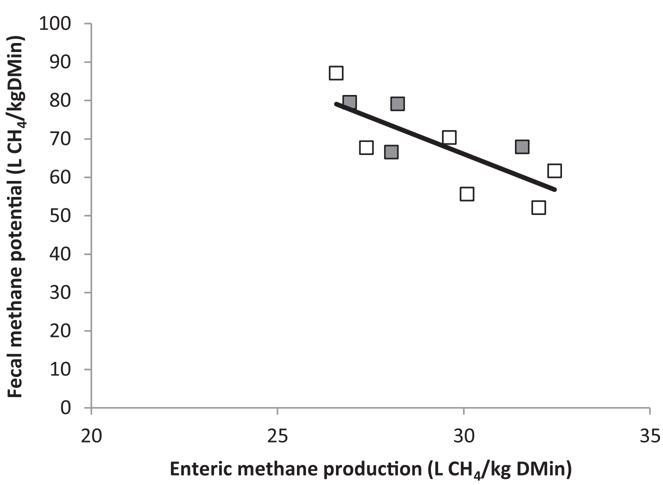

Fig. 2. Relationship between enteric methane production and ultimate methane yield (both expressed as L CH_4 per kg DM intake) using data from experiment 1 (filled data labels) and experiment 2 (unfilled data labels) ($y = -3.80x + 180.05$, $R^2 = 0.57$).

yields increased by 25–31% by a fat addition to diets. It was possible to predict the BMP value based on feed and feces characteristics. The best prediction was obtained by including both feed and feces characteristics. There was a strong negative correlation between enteric CH_4 emission and B_0 in terms of DM intake by dairy cows, meaning that if enteric emissions are reduced by extra fat addition the subsequent manure storage potentially will lead to higher emissions unless proper manure handling as anaerobic digestion is included. Including anaerobic digestion, reduction in enteric emissions will lead to higher energy yield from biogas production, thus resulting in an additional gain in climate effect and economic benefits to farmers producing biogas trough anaerobic digestion.

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